November 27, 2000

**CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 00-16**

Subject: Issuance of New Supplemental Assay Method

To: Biologics Licensees, Permittees, and Applicants
Veterinary Services Management Team
Directors, Center for Veterinary Biologics

The following Supplemental Assay Method (SAM) has been approved:

STSAM0928.01 Supplemental Assay Method for Detection of Extraneous Bacteria and Fungi in Live Bacterial Vaccines. This SAM is new and describes the test procedure used to detect viable extraneous bacteria and fungi in live bacterial vaccines. If extraneous viable bacteria or fungi are present, they will be detected by morphology, Gram stains, differential agar, and agar slants when compared to appropriate positive control. A draft of this SAM was circulated to CVB staff and biologics licensees, permittees, and applicants; the following comments were received. Changes made to the SAM, or additional information in response to the comment, are noted by comment.

**Comment 1:** Comment on **Section 4.10** - Instead of subculturing the "first" 3 tubes, it seems more appropriate to pick tubes 1, 3, and 9 or do a random pick.
**Response 1:** We chose to accept this comment and have changed **Section 4.10** to read "subculture 3 tubes, picked at random, from each temperature."

**Comment 2:** In **Section 3.3.3**, it should not be necessary to purity test the diluent more than once. This section implies that diluent testing is conducted each time product testing is conducted.
**Response 2:** We agree that the quality control (QC) departments at other laboratories may conduct their testing in different ways. This SAM explains how the test is conducted at the CVB-L. The CVB-L uses the firm's diluent to rehydrate live bacterial products whenever it is supplied. If it is not supplied, the CVB-L rehydrates with sterilized water. This section indicates that this water must be tested as a control for the test. Biologics firms are required to test lots of diluent according to the Code of Federal Regulations, Title 9 (9 CFR), Part 113.26.

**Comment 3:** Comment on the test being excessive when identity testing is already being conducted on the avirulent product organism.
**Response 3:** This purity test is not meant to duplicate the identity test of the product organism, although most will grow in the media used. This purity test is meant to identify those

serials of product which also contain contaminating bacteria and fungi along with those product organisms.

**Comment 4:** Comment that the live anthrax vaccine should be excluded from this test at this time.
**Response 4:** We disagree with this comment. The objective of this test method is to detect extraneous contamination in all live bacterial products.

**Comment 5:** Comment that 20 test vessels for technique controls in **Section 3.3.3** is redundant, expensive, and significantly increases testing time.
**Response 5:** We agree with this comment. The technique controls are not required by the 9 CFR. This section is an explanation of the way that the CVB-L technicians do this test. The CVB-L feels that technique controls need to be done by our laboratory to show that the water used as diluent, the technicians' technique, and the equipment (pipettes, syringes, etc.) have not led to an unsatisfactory test result for a firm's product.

**Comment 6:** Comment that the 20 test vessels required in **Section 3.3.4** is excessive and suggests that 1 media (negative) control is sufficient to confirm sterility.
**Response 6:** We agree that 20 test vessels (10 at each incubation temperature) is a large number. The 9 CFR, Part 113.25(c), requires that an adequate number of test vessels must be done to confirm sterility. The CVB-L uses media prepared fresh for each test session, and the sterility of the media batch is confirmed at the same time as the test. This is done by incubating 10 test vessels of thioglycollate medium at 30°-35°C and 10 test vessels of trypticase soy broth at 20°-25° C. Since the number that is considered "adequate" is not spelled out in the 9 CFR, each QC lab is free to determine their own media controls.

**Comment 7:** Comment questions the need for the positive control tubes specified in **Section 4.6**. Comment suggests it is unlikely that one turbid vessel will look much different than another and the subculturing will be necessary for confirmation anyway.
**Response 7:** The 9 CFR does not require that positive controls be done; they are just another tool the CVB-L uses to evaluate these live bacterial products for purity. Historically, the positive control tubes have helped us in our conclusions on unsatisfactory products. Although in some cases the macroscopic comparison has been helpful, the positive control is the most help through a comparison of the Gram stains or the colony morphology of the subcultures on differential agar.

**Comment 8:** Comment on **Section 4.9** on whether additional growth promotion is required at the time of testing, as long as the growth promotion has been checked at the time of manufacture.
**Response 8:** This is only a general comment that growth promotion testing is required on each batch of prepared media. This SAM serves as a guideline to the CVB-L technicians and instructs them to do growth promotion on the batch of media, which in CVB-L's case is usually made as a batch specifically for this purity testing.

**Comment 9:** Comment on **Section 4.10** suggests that all 20 of the test vessels should be subcultured, not just 3 out of 10 at each temperature of incubation. If a true contaminant is suspected or detected, a proper identification to genus would be useful.
**Response 9:** We agree that subculturing all 20 test vessels would be the ideal situation. However, it has been the CVB-L's experience that when live bacterial products have been contaminated, either they have macroscopically observable differences between test vessels, or all tubes contain the contaminant and the subculturing of 3 tubes has been sufficient. All 20 tubes are subcultured and/or Gram stained whenever a contaminant is found in any tube. The CVB-L has not needed identification to genus but it could be used as part of the subculture if macroscopic growth, Gram stain, colony morphology, and retesting are not sufficient.

**Comment 10:** Comment that **Section 5.1** must be a misprint because products and controls would exhibit growth since they are live bacterials.
**Response 10:** **Section 5.1** was put in the SAM to cover those live bacterial products that do not grow in trypticase soy broth and thioglycollate media. Mycoplasma vaccine is one example. Subculturing of these vaccines with no growth is not required, and results can be entered after 14 days of incubation.

This SAM is also available as an Adobe Acrobat pdf file on the world wide web (WWW) at http://www.aphis.usda.gov/vs/cvb/lab.

For those firms and interested parties with E-mail addresses and WWW access to the SAMs, this notification has been sent via electronic mail. If you would prefer to receive information from the Center for Veterinary Biologics via electronic mail, please send your E-mail address to cvb@usda.gov.

/s/ Randall L. Levings

Randall L. Levings
Director
Center for Veterinary Biologics

APHIS:VS:CVB:L:GChristianson:jed:ext.7416:10/26/00:f:/cvbl-com/Notice0016.lwp